Reference: *Biol. Bull.*, **150**: 241–254. (April, 1976)

HEART AND SCAPHOGNATHITE RATE CHANGES IN A EURYHALINE CRAB, *CARCINUS MAENAS*, EXPOSED TO DILUTE ENVIRONMENTAL MEDIUM

RICHARD I. HUME AND ALLAN BERLIND

Department of Biology, Wesleyan University, Middletown, Connecticut 06457

The mechanisms by which some crustaceans permanently or temporarily maintain their body fluids at osmotic or ionic concentrations different from those of their environment include the active transport of ions across the gills and other surfaces (Krogh, 1938; Green, Harsch, Barr, and Prosser, 1959; Shaw, 1961a), the limitation of diffusive permeability to water and ions of their surfaces (Smith, 1967, 1970; Shaw, 1961b; Rudy, 1967; Potts and Parry, 1964) and the control of the volume and/or ionic concentration of the urine (Shaw, 1961a; Green *et al.*, 1959). Some or all of the above processes appear to be controlled by hormonal mechanisms, especially in the case of crustaceans living in environments which vary in salinity. Although the physiological role of putative hormonal factors in hydromineral balance remains unclear in most cases, it is likely that endocrine factors from the eyestalk can effect the movement of sodium or water across the surface membranes in crabs and crayfish (Carlisle, 1955; Kamemoto, Kato, and Tucker, 1966; Kato and Kamemoto, 1969; Heit and Fingerman, 1975). Extracts of the thoracic ganglion and brain (which contain neurosecretory cell bodies) have likewise been found to alter the state of hydromineral balance or the movement of molecules when injected into whole animals (Kamemoto and Tullis, 1972) or when tested on isolated tissues (Mantel, 1968).

Tullis and Kamemoto (1974) have recently presented evidence for the presence in the thoracic ganglion and brain of the brackish water crab *Thalamita crenata* of two factors which appear to alter the permeability of surface tissues to water without altering the flux of sodium. A water-soluble component of small molecular weight decreases the water influx through the gills, while an acetone-soluble fraction increases the permeability of the gills, and perhaps of the gut as well. It is possible, however, that the changes in permeability to water do not reflect actual changes in the properties of the epithelium, since the rate at which water can move passively into or out of an animal not in osmotic equilibrium with its environment might be altered if the rate or pattern of circulation of the hemolymph in exposed surfaces (especially the gills) is altered, or if the rate of irrigation of the gills changes (Smith, 1970). Cornell (1973, 1974) has commented briefly on the possibility that changes in heart rate might account for an observed change in water flux in a stenohaline crab *Libinia emarginata*. In this paper we report on changes in heart rate in a euryhaline crab *Carcinus maenas* when transferred to dilute medium and discuss briefly the controlling mechanisms for the heart rate changes.

MATERIALS AND METHODS

Adult specimens of *Carcinus maenas* were obtained from the Marine Biological Laboratory, Woods Hole, Massachusetts, or were collected locally along the Connecticut shore. The animals, which weighed between 20 and 70 grams, were kept in circulating artificial sea water at 15° C in an Instant Ocean tank. Before use in an experiment an animal was placed in a shallow dish of sea water and allowed to equilibrate for at least 24 hours at 22–24° C, the temperature at which experiments were carried out. Ahsanullah and Newell (1971) have reported that there is very little thermal acclimation of heart rate in *C. maenas* over a period of 21 days, and all experiments were performed within this time after animals were removed from the tank. The fluids in equilibration and experimental chambers were vigorously aerated before and during the experiments, but the same results were obtained even if the chambers were not aerated during the course of the experiment.

Heart rate was monitored in freely moving crabs by the use of implanted electrocardiogram electrodes, which consisted of a short length of silver wire passed through a lucite block. The uninsulated end of the wire was inserted through a hole in the carapace, and the lucite block glued into place with Oneida Instant Weld adhesive. The best recordings were obtained when two electrodes were placed on the dorsal midline, one directly over the heart and one slightly anterior to it. Amplified signals were displayed on a chart recorder. About 75% of the implants yielded usable results.

The rate of scaphognathite beating was monitored in freely moving crabs by placing a cannula into the gill chamber through a hole in the dorsal surface. The cannula was attached to a pressure transducer, the output of which was amplified and displayed on a chart recorder. Most records were taken from the anterior portion of the epibranchial chamber.

Experiments were performed in smooth-sided chambers of white translucent plastic with snap-on lids. Smooth walls prevented the crabs from climbing out of the water, which they often attempted to do in response to being placed in highly diluted sea water. The use of translucent material minimized spontaneous heart stoppage and ventilation arrests which are often observed in response to visual stimuli. The chambers could be drained from the bottom, with fresh medium added rapidly from the top. All experiments were performed during natural daylight hours.

Artificial sea water was prepared by dissolving Instant Ocean sea salts in tap water. Dilutions were made by adding measured volumes of water to already mixed 100% SW, and are expressed as % SW. All solutions were vigorously aerated before being introduced into the chamber.

RESULTS

General characteristic of electrocardiogram records

Recorded heart rates varied markedly from animal to animal. Large crabs in general had slower rates than smaller crabs under the same conditions, as has been reported previously (Ahsanullah and Newell, 1971), and crabs that were monitored at lower temperatures had lower average frequencies (Florey and Kreibel, 1974). These overall trends were expected and were not extensively re-investigated, for it was found that individual variation of frequency of beat was considerable even after factors of size and ambient temperature were taken into account. The extent of the variability can be seen in Table I, where the five crabs numbered 1–5, all weighing between 30 and 32 grams, had heart rates at the beginning of their experimental runs which ranged from 101 to 184 beats/minute. In addition, there were often large differences in basal rate in the same crab from day to day (*e.g.*, for crabs 1, 5, 13 in Table I, compare the average starting rates on the control and experimental runs, which were performed on different days). The source of this variability is not clear. Apparently it is not simply due to the differences in general level of activity of the animal. Although rapid fluctuations in rate were often observed during periods of movement, the basal frequency normally returned rapidly to its previous level after the animal became quiet. In all of the records tabulated, readings were taken during prolonged periods during which there was no significant movement.

The heart frequency usually showed large, rapid and irregular changes when the medium was changed, and for several minutes thereafter. These fluctuations, which were not consistent in direction, were observed both when the salinity was changed and when the medium was simply renewed at the original salinity after the chamber was drained. Such fluctuations normally disappeared within a few minutes and only readings taken at least 20 minutes after a transfer were used in analyzing the results of these experiments.

Responses of the heart to dilute external medium

In this set of experiments, the medium was changed from 100% SW to 15% SW and then back to 100% SW. In control runs the same animals (on different days) were subjected to fresh 100% SW during the middle period. In each of 13 experimental runs of this type, the heart rate markedly increased when the animal was transferred to 15% SW. The average increase was 51 beats/minute, or 46%. These changes were observed whether transfer was accomplished by draining the tank completely and adding fresh 15% SW, or by adding the appropriate volume of tap water to 100% SW. When the animals were transferred from 100% SW to fresh 100% medium, only very small changes (increases or decreases) were observed, (averaging − 1 beat/minute, or − 1% from the basal level). The complete set of data is provided in Table I.

Results from a typical experiment are shown in Figure 1. The heart in most cases took about 30 minutes to reach its maximum, after which its rate stayed fairly constant for at least two hours. An increase was often noted within the first 10 minutes after transfer. Whether the response actually began before this point is not clear because of the fluctuations induced by the transfer. When the crab was returned to 100% SW after two hours in 15% SW, the heart rate gradually returned to about the level that it had shown initially in 100% SW. The time course of the return was quite variable. In some cases the frequency returned to the basal level by 18–20 minutes after transfer but more typically took over half an hour to come down.

TABLE I

Effects on heart rate of transferring Carcinus *from 100% SW to 15% SW (experimental) or from 100% SW to fresh 100% SW (control).*

Crab (Arranged by weight)	Sex	Weight (grams)	Experimental (100% SW → 15% SW)				Control (100% SW → 100% SW)			
			Average rate before (beats/min)	Average rate after	Net change	Percent change	Average rate before (beats/min)	Average rate after	Net change	Percent change
1	F	30	125	202	+77	+62%	197	188	−9	−5%
2	M	31	153	202	+49	+32%	159	157	−2	−1%
3	M	32	181	206	+25	+14%	194	195	+1	+1%
4	M	32	184	211	+27	+15%	161	162	+1	+1%
5	M	32	101	205	+104	+103%	190	196	+6	+3%
6	M	35	137	197	+60	+44%	148	149	+1	+1%
7	F	39	186	209	+23	+12%	191	186	−5	−2%
8	F	46	158	204	+46	+29%	159	156	−3	−2%
9	M	49	175	190	+15	+9%	175	177	+2	+1%
10	F	49	107	142	+35	+33%	74	68	−6	−8%
11	F	49	97	144	+47	+48%	109	123	+14	+13%
12	F	55	78	136	+58	+74%	104	95	−9	−9%
13	F	55	75	167	+92	+122%	38	36	−2	−5%
Means ±s.e.			135 ± 11	186 ± 8	+51 ± 8	46 ± 10%	146 ± 14	145 ± 14	−1 ± 2	−1 ± 2%

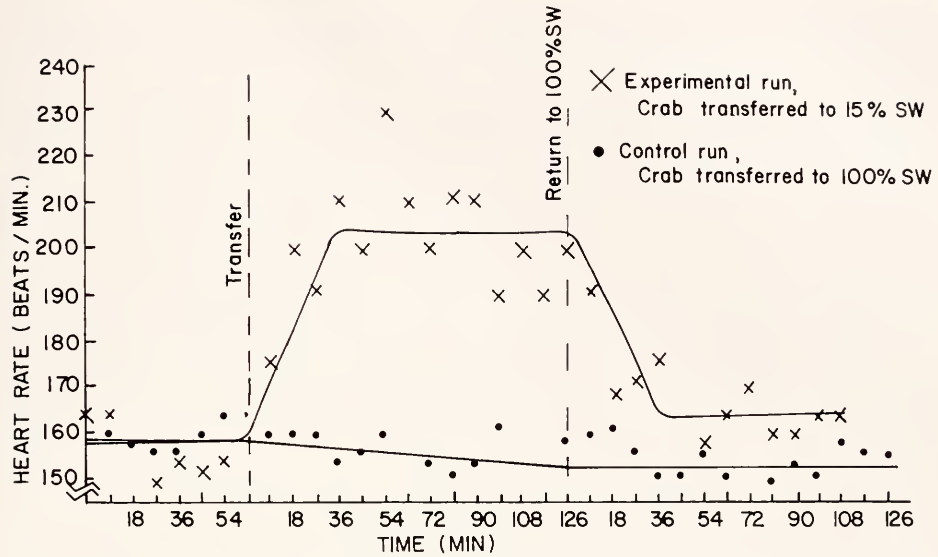

FIGURE 1. Heart rate of one *Carcinus maenas* during a control run (transfer to fresh 100% SW) and an experimental run (transfer to 15% SW). The two trials were run on different days. The results of this experiment are typical in terms of the increase of frequency (pattern, time course, and magnitude of the increase), but somewhat atypical in that both the control and experimental runs started at almost the same basal frequency.

In several of the experiments described below, in which smaller numbers of animals were used, the average basal heart rate before transfer to an experimental medium was considerably higher than that of the large group tabulated here. If the heart responses of all animals from Table I with a basal rate of 150 or higher

TABLE II

Effects on heart rate in Carcinus *of various experimental conditions.*

Experiment	External medium during test period	Conditions	Number of animals	Average rate before transfer (beats/min)	Average rate after transfer (beats/min)	Net change	Percent change
A	25% SW isosmotic with SW	sucrose added to maintain osmolarity	7	122 ± 10	152 ± 8	+30	+25%
B	low sodium	choline substituted for 75% of sodium	4	158 ± 25	147 ± 24	−11	−7%
C	low chloride	acetate substituted for 75% of chloride	4	174 ± 15	174 ± 13	0	0
D	15% SW	eyestalkless	5	146 ± 10	179 ± 9	+33	+23%
E	15% SW	all animals from Table 1 with a "before" rate of 150 or greater	6	173 ± 6	204 ± 3	+31	+18%

are analyzed as a separate group, a marked cardioacceleration is still observed after transfer to 15% SW (Table II, line E).

Relation between salinity and heart rate

By adding measured amounts of water to a known volume of 100% SW, the salinity in an experimental chamber was lowered in a number of steps. The crab was left in each solution for one hour, and the mean rate was calculated from the readings taken from 20 to 60 minutes after transfer. The averaged results from experiments on ten crabs are shown in Figure 2. From 100% SW down to about 75% SW, there was little or no cardioacceleration with decreasing salinity. Beyond this point, the rate increased as the salinity was decreased.

To insure that the order of exposure to different media did not account for the results, several animals were transferred directly from 100% SW to 15%, and then to one intermediate dilution (usually 40% or 50%) before being returned to 100% SW. In four animals tested, the heart rate was fastest in the most dilute medium and intermediate in the middle dilution.

Effect of osmotic concentration of the external medium on heart rate

To determine whether the cardioacceleration response is attributable to the lowered osmotic strength of dilute sea water, crabs were transferred to solutions

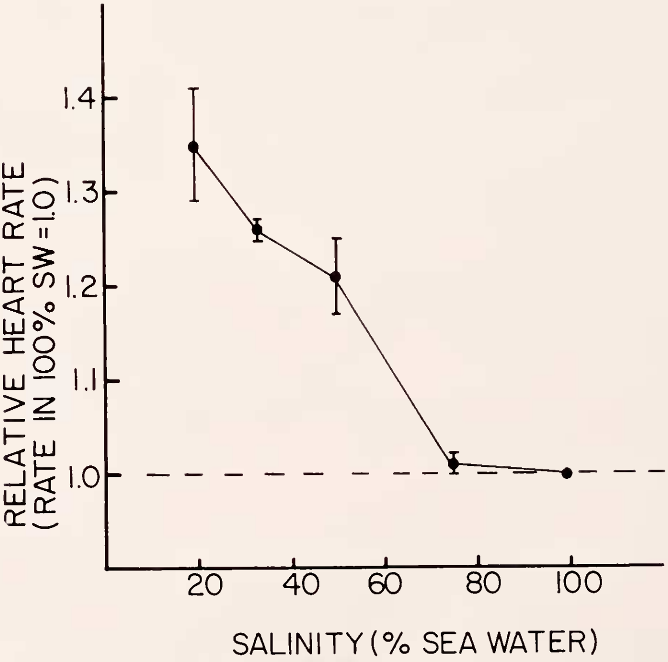

FIGURE 2. Heart response in *Carcinus maenas* as a function of salinity of the external medium. The vertical axis shows the ratio of the heart rate in diluted medium to that in 100% SW (mean value and standard error from experiments on ten animals). The nature of the curve is similar whether the salinity is reduced stepwise or the animal is transferred directly from sea water to the most dilute medium and then to intermediate salinities.

which were isosmotic with 100% SW but had only 25% of the ions found in normal sea water. Solutions were made isosmotic with sea water by adding an appropriate amount of sucrose to 25% SW. When transferred into the test medium, six of the seven animals tested showed a heart acceleration response similar to animals transferred to 25% SW (hyposomotic). The averaged results for all seven animals are shown in Table II, line A.

Effects of alterations of sodium and chloride concentrations on heart rate

The results cited above suggest that osmotic strength of the medium is not the parameter on which the cardioacceleratory response is based. It is possible that the crab perceives salinity changes by monitoring the concentration of one of the major ionic constituents of sea water. The effects of altering the concentration of specific ions was examined. One modified saline contained only 15% sodium but nearly normal chloride, and another 15% chloride but nearly normal sodium. The two modified media were prepared by adding 150 ml of 100% SW to each 850 ml of isotonic choline chloride in the first case, and 850 ml of isotonic sodium acetate in the second. The minor ionic constituents were therefore at 15% of their sea water concentrations in both modified media. Four crabs were tested in each altered sea water.

Neither ionic modification caused an acceleration of heart rate. When crabs were transferred from normal sea water to a medium with normal sodium but low chloride, the heart frequency was essentially unchanged (Table II, line C). In the normal chloride medium with low sodium, the rates in all four cases were slightly decreased (Table II, line B). Since no cardioacceleration was observed with either modification, and all other ions were low in both altered media, it is unlikely that the concentration of any specific ion in the external medium is the controlling parameter for the heart response.

Site of the receptors

In an attempt to localize the receptors for salinity changes a special chamber was constructed which allowed the front of the crab (with its mouthparts, antennules and antennae) to be exposed to one salinity, while the rear of the crab (including the gill chamber intake areas) could be exposed to different medium. Th crab was placed in the opening of an impermeable, tightly fitting rubber membrane (the neck of a rubber balloon) which was used to separate the two ends of a small lucite chamber. Each section of the chamber had an inflow and an outflow spigot which allowed independent perfusion of the two compartments. Both chambers were continuously perfused to prevent the accumulation of wastes or the depletion of oxygen. Since the incurrent openings of the gill cavity were in the rear chamber and the excurrent openings in the front, the animal tended to transfer the rear medium into the front chamber during normal pumping. To minimize the effects of such transfer, perfusion rates of the two chambers were rapid. It is likely, however, that particularly in the areas around the excurrent openings, the crab was exposed to a salinity intermediate between those stated for the front and rear chambers.

TABLE III

Effects on heart rate of independent perfusion of anterior and posterior ends of crabs.

Crab	Front in: 100% SW Back in: 100% SW		100% SW 15% SW	15% SW 15% SW	15% SW 100% SW
A	Rate	174 ± 2	188 ± 2	204 ± 2	188 ± 2
B	Rate	184 ± 2	181 ± 3	194 ± 1	184 ± 3

The experiments were performed by (1) first perfusing both chambers with 100% SW; (2) switching one chamber only to 15% SW; (3) perfusing both ends of the crab with 15% SW; (4) reversing the situation from that in step (2). The only firm conclusion that can be drawn from experiments on eight animals is that a maximal increase in heart rate occurs only if both ends of the crab are bathed in dilute medium. In some cases, slight increases in heart frequency were observed when one end or the other was in 15% SW (*e.g.*, crab A in Table III), while in other trials, no increase at all was seen unless both ends were in dilute medium (*e.g.*, crab B). The response to complete exposure to 15% SW was similar to that in the basic experiments described earlier despite the fact that the animals were immobilized by the membrane and had the claws removed.

In a second series of experiments suspected chemoreceptor structures were removed from several crabs. The antennules and antennae are known to contain chemoreceptors, which respond differentially to changes in external salinity in a number of crustaceans (van Weel and Christofferson, 1966; Lagerspetz and Mattila, 1961; Tazaki, 1975). Antennules were removed from four crabs. In all four animals the cardioacceleratory response to dilute saline persisted. Surgical removal of the antennae likewise did not eliminate the response in four animals, nor did the elimination of the chelae.

Effect of eyestalk removal

The neurosecretory center in the eyestalks of crustaceans is likely to exert some control over salt and water balance. In addition, some factor(s?) present in the X organ-sinus gland complex can apparently speed up isolated decapod hearts (Welsh, 1937; Knowles and Carlisle, 1956). Eyestalks were therefore removed from five crabs, to determine whether a hormonal factor from the neurosecretory complex might be involved in the excitatory response to dilute medium. In all five animals the heart frequency increased when the animal was placed in 15% SW, despite the absence of eyestalks (Table II, line D).

Effects of dilute medium on scaphognathite rate

Ten crabs with a gill chamber cannulated were transferred from 100% SW to 15% SW for one to two hours and then returned to sea water. In six of these animals the scaphognathite rate was markedly *decreased* when the animal was in dilute medium. The scaphognathite not only beat more slowly, but in several cases, intermittantly stopped beating for periods of up to several seconds (Figure 3). In the other four animals, no consistent changes in rate in either direction were noted. None of the animals transferred to fresh 100% medium showed consistent changes in scaphognathite rate.

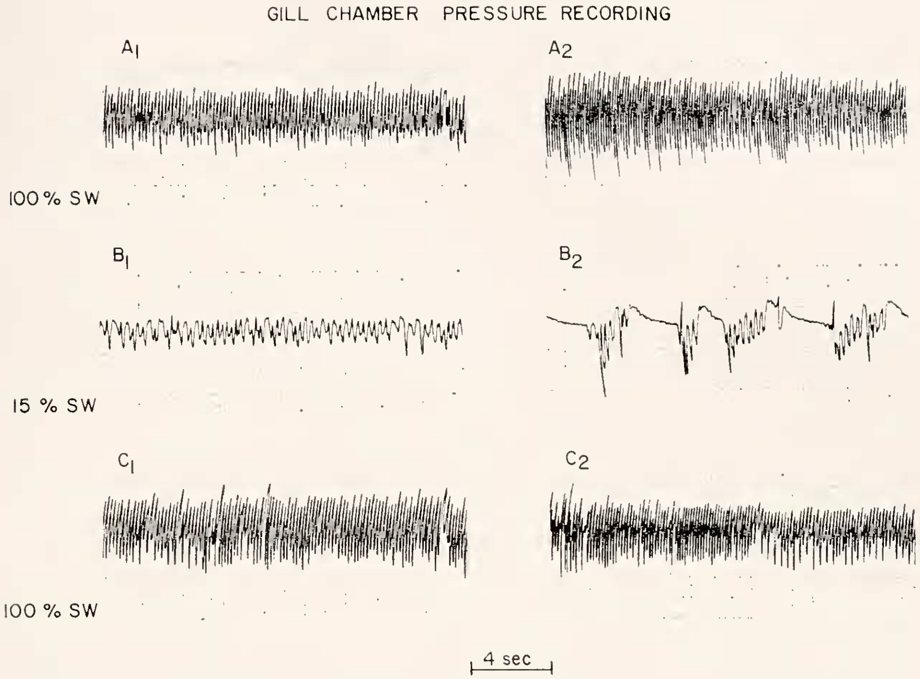

FIGURE 3. Pressure transducer records from the epibranchial cavity of *Carcinus* during exposure to normal and diluted sea water. A), pretest in 100% SW; B), exposure to 15% SW; C), retest in 100% SW. In each case, the right tracing was taken later than that on the left. The spontaneous stoppages of beating seen in 15% SW (B_2) were almost never seen in 100% SW, although arrests can be induced by visual, tactile, or auditory disturbances.

DISCUSSION

A marked increase in heart rate is observed in the euryhaline decapod *Carcinus maenas* when the animal is transferred from 100% SW to a more dilute medium. Cardioacceleration is not observed until the environmental medium falls below 75% SW. *Carcinus* is an osmoconformer between 100% and 75% SW and begins to hyperregulate only when the external salinity falls below this level (Shaw, 1961a). It therefore seems likely that cardioacceleration is part of an adaptive regulatory response to dilute medium.

Similar heart responses have been reported previously in representatives of two other groups of crustaceans, the euryhaline amphipod *Gammarus duebeni* (Kinne, 1952), and in the euryhaline shrimp *Crangon crangon* (Spaargaren, 1973). In the stenohaline crab *Libinia emarginata,* however, contrasting results have been reported. Cornell (1973, 1974) has reported a decrease in heart rate when individuals of this species are transferred from 100% SW to 80% SW, which is near the lower limits of its tolerance. Cornell has hypothesized that a decrease in heart rate might account for the decrease in water permeability of the body surface that is observed in some crabs when they are placed in a medium which differs in osmotic concentration from that of their body fluids. If a decline in the heart rate results in a corresponding decrease in the flow of hemolymph through the gills, thus reducing the average gradient for the inward movement of water, a decrease in water flux could result without a real change in the properties of the gill epithelium. Our results suggest that in euryhaline crustaceans different mechanisms operate.

Interpretation of the adaptive value of an increase in heart rate depends on a knowledge of the cardiac output of the heart. It is generally assumed that as the frequency of beat increases cardiac output also increases. Florey and Kreibel (1974), have suggested from studies in *Cancer magister*, that increased heart rate might, under some conditions, lead to a decrease in cardiac output due to incomplete filling of the heart at each beat. This conclusion, based on calculations from respiratory data of Johansen, Lenfant and Mecklenberg, (1970) depends on the extremely large temperature coefficient of the p50 of crab hemocyanin. If, in *Carcinus* held at a constant temperature, heart rate increase results in decreased cardiac output, the correspondingly lower flow of hemolymph through the gills might directly account for the observed decline in "water permeability". If, on the other hand, cardiac output increases with increased rate, the adaptive value of the observed response might be related to one or more of several physiological factors involved in hyperosmotic regulation: 1), an increased flow of hemolymph through the gills might be a mechanism to facilitate oxygen uptake, which increases markedly in *Carcinus* in dilute medium (Potts and Parry, 1964); 2), an increased cardiac output could lead to more effective dispersion of metabolic substrates and hormones involved in regulation; and/or 3) the volume of urine produced by *Carcinus* increases by a factor of about ten when the animal is transferred from 100% SW to 40% SW (Shaw, 1961a). Urine is apparently formed by a filtration mechanism (Binns, 1969; Lockwood and Inman, 1973; Riegel, Lockwood, Norfold, Bulleid, and Taylor, 1974), and an increase in heart output might enhance filtration. A more detailed discussion of the possible significance of the heart rate increase awaits the availability of detailed information on cardiac output as a function of heart rate in *Carcinus* at constant temperature.

We have not yet delineated the pathways by which salinity changes in the external medium exert a control over heart rate. Our attempts to localize receptors for the salinity change have yielded inclusive results. It is possible that internal salinity receptors are involved, but we do not consider this likely. The internal osmotic concentration of *Carcinus* falls only very slowly (Margaria, 1931; Shaw, 1961a) and reaches its lowest levels only after 12–15 hours of exposure of the animal to dilute medium. After one to two hours of exposure to 33% SW or 25% SW, the internal concentration drops by less than 10%, and is still far above the final level reached. In addition, the rate of fall of internal concentrations appears to be similar when the animal is exposed to a range of external salinities below 50% (Margaria, 1931). The heart response reported here levels off after 10–30 minutes exposure to dilute medium despite the fact that the internal salinity is still falling. In our experiments where animals were transferred from 100% SW to 15% SW for a short period, and then placed at an intermediate salinity (40% or 50% SW), the heart rate was faster in 15% SW than in the intermediate salinity. Since the internal salinity is continuously falling throughout the course of this experiment, it appears that the heart rate is more closely linked to an external factor than to any internal one.

The observation that a maximal response is observed only when both ends of the animal are exposed to dilute medium might imply that cooperative interaction between receptors in different areas (*e.g.*, anterior appendages and gill chambers) is involved. Alternatively, receptors in the region of the mouthparts or the exhalant openings of the gill chambers may mediate the response. Salinity-sensitive cells in either of these areas might be influenced by the solutions in both chambers in our experimental design, since fluid from the rear chamber is pumped forward by the animal over these sites. The antennules, antennae and chelae have been eliminated as the only receptor sites mediating the response (despite the presence of salinity receptors on some of these appendages) since their ablation does not alter the reults of transfer to 15% SW.

The output pathway of the heart regulatory system in the context of osmotic regulation is likewise not yet determined. A direct effect of dilution of the internal fluids on heart function appears to be ruled out for the same reason that internal receptors were eliminated from consideration. The heart is subject to excitatory control by regulatory axons which synapse on the cardiac ganglion cells, and possibly by hormones from several sources (Maynard, 1960; Knowles and Carlisle, 1956). Cardioexcitor factors, which can be released from neurosecretory endings into the bathing medium and are presumably hormonal in nature, are found in the pericardial organs and in the sinus gland-X organ system. The physiological conditions under which these possible cardioexcitor hormones are released into the circulation in intact animals are almost completely unknown. The sinus gland has been eliminated as a source of cardioexcitor hormone in osmoregulatory adjustments, since extirpation of the entire eyestalk does not abolish the response. We are currently investigating the possible role of pericardial organs as part of the pathway by which external salinity changes cause changes in heart rate. There have been reports of differences in relative weights of pericardial organs in euryhaline as compared to stenohaline species of crabs (Maynard, 1961), but no information has been published to date that indicates differences in cardioexcitor content or secretion rates.

The striking similarities between the control of the cardioacceleratory response reported here and the permeability changes described by Smith (1967, 1970) and Capen (1972) in the euryhaline *Rhithropanopeus* and *Carcinus* are worth noting. Smith found that no significant permeability change occurs in *Carcinus* until the medium drops below 75% SW; there is also no cardioacceleration down to this level. In *Rhithropanopeus*, water permeability decreases in dilute sea water made isosomotic to 100% SW by the addition of sucrose while no permeability changes are observed in low Na^+ or low Cl^- sea waters; the heart rate changes described here show the same osmotic and ionic dependencies. Removal of antennules does not effect permeability changes in *Rhithropanopeus* nor does it effect the cardioacceleratory response in *Carcinus*. The heart response appears to reach its peak more rapidly than does the water permeability change. This may in part be due to the nature of the recording techniques, which allow an analysis of events occurring over a shorter time course in the case of heart rate, but it may also represent a real difference in the output end of the system. Capen's experiments do not distinguish between hormonal and nervous nature of the output, nor do ours, but the similarities of the two responses suggest that the heart rate changes and the permeability changes are parts of the same adaptive mechanisms which are induced to cope with a state of hydromineral imbalance.

A sharp decrease in scaphognathite rate occurred in more than half of the crabs transferred to a dilute medium. The variability in the time course of the drop in frequency in responding animals was much greater than it was for the heart rate change; in some cases the scaphognathite rate dropped off within a minute after transfer, but in others the rate was maintained at its previous level for half an hour or more before starting to decline. If a decrease in scaphognathite rate is a mechanism for limiting the exchange of material across the gills, it is not clear why the remaining animals gave no response at all. Arudpragasam and Naylor (1964a, b) have argued that the periodic reversals of scaphognathite beating may be functionally important in ventilation, and that the frequency of beating of the appendage during normal forward pumping may not be an accurate parameter of the potential for exchange. These conclusions have been challenged on the basis of careful measurements of pO_2 and hydrostatic pressure changes in different regions of the gill chamber during reversal (Hughes, Knights, and Scammel, 1969). In our experiments there were no consistent changes in the frequency of reversals of the pump, either in animals which showed a marked decline in the rate of forward beating, or in animals which retained the normal rapid forward pumping in dilute medium.

There have been numerous previous reports of coordinated changes in heart and scaphognathite rate in several crustaceans. Low oxygen content in the medium induces a simultaneous slowdown in lobsters (McMahon and Wilkens, 1972). In *Carcinus* the same treatment slows the heart and has little effect on the frequency of forward pumping, but may alter the nature of reversals of the scaphognathite (Taylor, Butler, and Sherlock, 1973). A variety of tactile and chemical stimuli can cause reflex changes in the two systems in the same direction [CO_2, N_2, NaCl and sugars cause slowdown or stoppages of both in the crayfish (Larimer, 1964; Ashby and Larimer, 1965)]. Conversely, jets of water cause a decrease in heart rate and an increase in ventilation in crayfish (Larimer, 1964). Wilkens, Wilkens, and McMahon (1974) have reported the presence of interneurons in the connectives of the crab *Cancer magister* which alter the function of both systems. In addition, factor(s?) contained in a crude extract of pericardial organs speed up scaphognathite rate as well as heart rate (Berlind, unpublished). Very few naturally occurring stiumli cause an increase in heart rate. It is clear that, while heart and ventilation responses are often coupled, they are to some degree subject to independent control or can change in opposite directions in response to the same stimulus. It is of interest with regard to this point that 5 hydroxytryptamine, a suspected neurotransmitter or neurohormone in crabs speeds up the heart but slows the scaphognathite (Berlind, unpublished). The control mechanisms which account for the different responses or heart and scaphognathite in the context of osmoregulation remain to be determined.

We would like to thank Denise Lensing and Susan Feinstein for assistance with some of the experiments, and F. I. Kamemoto, R. I. Smith, and S. J. Berry for criticizing the manuscript. The research reported was supported by a grant from NIH (Neurological Diseases and Stroke #NS 11163–01).

SUMMARY

When the euryhaline decapod *Carcinus maenas* is transferred from sea water to a medium more dilute than 75% sea water, the frequency of heart beat increases. The cardioacceleratory response is probably mediated by external rather than internal receptors, and the parameter that is sensed in neither the total osmotic strength of the medium, nor the concentration of any single ion. Although it is not yet determined whether the heart responds to a hormonal or nervous signal, it is likely that the control mechanisms are similar to those which mediate the water permeability changes in *Carcinus* and other euryhaline crabs.

In somewhat more than half of the crabs tested, the frequency of scaphognathite beating is markedly reduced when the animal is exposed to dilute external medium.

LITERATURE CITED

AHSANULLAH, M., AND R. C. NEWELL, 1971. Factors affecting the heart rate of the shore crab *Carcinus maenas* (L.). *Comp. Biochem. Physiol.*, **39A**: 277–287.

ARUDPRAGASAM, K. D., AND E. NAYLOR, 1964a. Gill ventilation and role of reversed respiratory currents in *Carcinus maenas* (L.) *J. Exp. Biol.*, **41**: 299–307.

ARUDPRAGASAM, K. D., AND E. NAYLOR, 1964b. Gill ventilation volumes, oxygen consumption and respiratory rhythms in *Carcinus maenas* (L.). *J. Exp. Biol.*, **41**: 309-321.

ASHBY, E. A., AND J. L. LARIMER, 1965. Modification of cardiac and respiratory rhythms in crayfish following carbohydrate chemoreception. *J. Cell Comp. Physiol.*, **65**: 373–379.

BINNS, R., 1969. The physiology of the antennal gland of *Carcinus maenas* (L.). I. The mechanism of urine production. *J. Exp. Biol.*, **51**: 1–10.

CAPEN, R. L., 1972. Studies of water uptake in the euryhaline crab, *Rhithropanopeus harrisi*. *J. Exp. Zool.*, **182**: 307–320.

CARLISLE, D. B., 1955. On the hormonal control of water balance in *Carcinus*. *Pubbl. Sta. Zool. Napoli.*, **27**: 227–231.

CORNELL, J. C., 1973. A reduction in water permeability in response to a dilute medium in the stenohaline crab *Libinia emarginata* (Brachyura, Majidae). *Biol. Bull.*, **145**: 430–431.

CORNELL, J. C., 1974. A reduction in water exchange rates in an osmoconforming crab. *Amer. Zool.*, **14**: 1259.

FLOREY, E., AND M. E. KRIEBEL, 1974. The effects of temperature, anoxia and sensory stimulation on the heart rate of unrestrained crabs. *Comp. Biochem. Physiol.*, **48A**: 285–300.

GREEN, J. W., M. HARSCH, L. BARR, AND C. L. PROSSER, 1959. The regulation of water and salt by fiddler crabs, *Uca pugnax* and *Uca pugilator*. *Biol. Bull.*, **116**: 76–87.

HEIT, M., AND M. FINGERMAN, 1975. The role of an eyestalk hormone in the regulation of the sodium concentration of the blood of the fiddler crab, *Uca pugilator*. *Comp. Biochem. Physiol.* **50A**: 277–280.

HUGHES, G. M., B. KNIGHTS, AND C. A. SCAMMELL, 1969. The distribution of PO_2 and hydrostatic pressure changes within the branchial chambers in relation to gill ventilation of the shore crab, *Carcinus maenas* (L.). *J. Exp. Biol.*, **51**: 203–220.

JOHANSEN, K., C. LENFANT, AND T. A. MECKLENBURG, 1970. Respiration in the crab, *Cancer magister*. *Z. Vergl. Physiol.*, **70**: 1–19.

KAMEMOTO, F. I., K. N. KATO, AND L. E. TUCKER, 1966. Neurosecretion and salt and water balance in the Annelida and Crustacea. *Amer. Zool.*, **6**: 213–219.

KAMEMOTO, F. I., AND R. E. TULLIS, 1972. Hydromineral regulation in decapod crustacea. *Gen. Comp. Endocrinol., Supplement* **3**: 299–307.

KATO, K. N., AND F. I. KAMEMOTO, 1969. Neuroendocrine involvement in osmoregulation in the grapsid crab, *Metopograpsus messor*. *Comp. Biochem. Physiol.*, **28**: 665–674.

KINNE, O., 1952. Zur Biologie und Physiologie von *Gammarus duebeni* Lillj. V. Untersuchungen über Blutkonzentration, Herzfrequenz und Atmung. *Kieler Meeresforsch*, **9**: 134–150.

KNOWLES, F. G. W., AND D. B. CARLISLE, 1956. Endocrine control in the crustacea. *Biol. Rev.,* **31**: 396–473.

KROGH, A., 1938. The active absorption of ions in some fresh water animals. *Z. Vergl. Physiol.,* **25**: 335-350.

LAGERSPERTZ, K., AND M. MATILLA, 1961. Salinity reactions of some fresh- and brackish-water crustaceans. *Biol. Bull.,* **120**: 44–53.

LARIMER, J. L., 1964. Sensory-induced modifications of ventilation and heart rate in crayfish. *Comp. Biochem. Physiol.,* **12**: 24–36.

LOCKWOOD, A. P. M., AND C. B. E. INMAN, 1973. Water uptake and loss in relation to salinity of the medium in the amphipod crustacean *Gammarus duebeni. J. Exp. Biol.,* **58**: 149–163.

MANTEL, L. H., 1968. The foregut of *Gecarcinus lateralis* as an organ of salt and water balance. *Amer. Zool.,* **8**: 433–442.

MARGARIA, R., 1931. The osmotic changes in some marine animals. *Proc. Roy. Soc. London Ser. B.,* **107**: 606–624.

MAYNARD, D. M., 1960. Circulation and heart function. Pages 161–226 in T. Waterman, Ed., *The Physiology of Crustacea,* Vol. 1. Academic Press, New York, New York.

MAYNARD, D. M., 1961. Thoracic neurosecretory structures in Brachyura. I. Gross anatomy. *Biol. Bull.,* **121**: 316–329.

McMAHON, B. R., AND J. L. WILKENS, 1972. Simultaneous apnoea and braycardia in the lobster, *Homarus americanus. Can. J. Zool.,* **50**: 165–170.

POTTS, W. T. W., AND G. PARRY, 1964. *Osmotic and ionic regulation in animals.* The Macmillan Co., New York, New York, 423 pp.

RIEGEL, J. A., A. P. M. LOCKWOOD, J. R. W. NORFOLK, N. C. BULLEID, AND P. A. TAYLOR, 1974. Urinary bladder volume and the reabsorption of water from the urine of crabs. *J. Exp. Biol.,* **60**: 167–181.

RUDY, P. P., 1967. Water permeability in selected decapod Crustacea. *Comp. Biochem. Physiol.,* **22**: 581–589.

SHAW, J., 1961a. Studies on ionic regulation in *Carcinus maenas* (L.). I. Sodium balance. *J. Exp. Biol.,* **38**: 135–152.

SHAW, J., 1961b. Sodium balance in *Eriocheir sinensis* (M. Edw.). The adaptation of the crustacea to fresh water. *J. Exp. Biol.,* **38**: 153-162.

SMITH, R. I., 1967. Osmotic regulation and adaptative reduction of water permeability in a brackish-water crab, *Rhithropanopeus harrisi* (Brachyura, Xanthidae). *Biol. Bull.* **133**: 643–658.

SMITH, R. I., 1970. The apparent water-permeability of *Carcinus maenas* (Crustacea, Brachyura, Portunidae) as a function of salinity. *Biol. Bull.,* **139**: 351–363.

SPAARGAREN, D. H., 1973. The effect of salinity and temperature on the heart rate of osmoregulating and osmoconforming shrimp. *Comp. Biochem. Physiol.,* **45A**: 773–783.

TAYLOR, E. W., P. T. BUTLER, AND P. J. SHERLOCK, 1973. The respiratory and cardiovascular changes associated with the emersion response of *Carcinus maenas* (L.) during environmental hypoxia at three different temperatures. *J. Comp. Physiol.,* **86**: 95–115.

TAZAKI, K., 1975. Sensory units responsive to osmotic stimuli in the antennae of the spiny lobster *Panulirus japonicus. Comp. Biochem. Physiol.,* **51A**: 647–653.

TULLIS, R. E., AND F. I. KAMEMOTO, 1974. Separation and biological effects of CNS factors affecting water balance in the decapod crustacean *Thalamita crenata. Gen. Comp. Endocrinol.,* **23**: 19-28.

VAN WEEL, P. B., AND J. P. CHRISTOFFERSON, 1966. Electrophysiological studies on perception in the antennulae of certain crabs. *Physiol. Zool.,* **39**: 317–325.

WELSH, J. H., 1937. The eyestalk hormone and rate of heartbeat in crustaceans. *Proc. Nat. Acad. Sci.,* **23**: 458–460.

WILKENS, J. L., L. A. WILKENS, AND B. R. McMAHON, 1974. Central control of cardiac and scaphognathite pacemakers in the crab, *Cancer magister. J. Comp. Physiol.,* **90**: 89–104.